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Acute administration of vinpocetine, a phosphodiesterase type 1 inhibitor, ameliorates hyperactivity in a mice model of fetal alcohol spectrum disorder☆

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ABSTRACT

Background: Maternal alcohol use during pregnancy causes a continuum of long-lasting disabilities in the offspring, commonly referred to as fetal alcohol spectrum disorder (FASD). Attention-deficit/hyperactivity disorder (ADHD) is possibly the most common behavioral problem in children with FASD and devising strategies that ameliorate this condition has great clinical relevance. Studies in rodent models of ADHD and FASD suggest that impairments in the cAMP signaling cascade contribute to the hyperactivity phenotype. In this work, we investigated whether the cAMP levels are affected in a long-lasting manner by ethanol exposure during the third trimester equivalent period of human gestation and whether the acute administration of the PDE1 inhibitor vinpocetine ameliorates the ethanol-induced hyperactivity.
Methods: From postnatal day (P) 2 to P8, Swiss mice either received ethanol (5 g/kg i.p.) or saline every other day. At P30, the animals either received vinpocetine (20 mg/kg or 10 mg/kg i.p.) or vehicle 4 h before being tested in the open field. After the test, frontal cerebral cortices and hippocampi were dissected and collected for assessment of cAMP levels.
Results: Early alcohol exposure significantly increased locomotor activity in the open field and reduced cAMP levels in the hippocampus. The acute treatment of ethanol-exposed animals with 20 mg/kg of vinpocetine restored both their locomotor activity and cAMP levels to control levels.
Conclusions: These data lend support to the idea that cAMP signaling system contribute to the hyperactivity induced by developmental alcohol exposure and provide evidence for the potential therapeutic use of vinpocetine in FASD.

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1. Introduction

Maternal ethanol use during pregnancy causes a continuum of long-lasting disabilities in the offspring (Riley and McGee, 2005) commonly referred to as fetal alcohol spectrum disorder (FASD). It is estimated that the prevalence of FASD in school children may be as high as 2–5% in developed countries (May et al., 2009). Several neurobehavioral problems can be observed in FASD (Kelly et al., 2000; Kodituwakku, 2009; Riley and McGee, 2005), and attention-deficit/hyperactivity disorder (ADHD) is possibly the most commonly observed behavioral problem (Bhatara et al., 2006; Burd et al., 2003; Doig et al., 2008). It was estimated that as many as 41% of children with FASD have a comorbid ADHD diagnosis (Bhatara et al., 2006), while in studies considering children with fetal alcohol syndrome (FAS), which represents the most severe outcome of prenatal ethanol exposure (Goodlett et al., 2005; Riley and McGee, 2005), this percentage ranges from 73% (Burd et al., 2003) to 95% (Fryer et al., 2007).

Although the three main symptoms of ADHD, impulsiveness, inattentiveness and hyperactivity, have been modeled in rodents (Sagvolden et al., 2005), hyperactivity is the most frequently studied by far. Murine hyperactivity has been usually assessed in the open field test, which estimates ambulatory movements on a wide surface. Despite its simplicity, the measure of ambulation has proven to be a useful tool in studies designed to predict aspects of behavior, genetics, and neurobiology of ADHD (Lalonde and Strazielle, 2009; Sagvolden et al., 2005). Locomotor hyperactivity is a pivotal behavioral trait observed in several inbred strains, knockouts, and transgenic rodents used as models of ADHD (Russell, 2007; Sagvolden et al., 2005). In FASD rodent models,

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locomotor hyperactivity has been consistently described in animals exposed to ethanol during the third trimester equivalent period of human gestation (Kelly et al., 1987; Melcer et al., 1994; Riley et al., 1993; Slawecki et al., 2004; Thomas et al., 2001, 2007), which, in mice and rats, corresponds to the first 10-day period after birth. During this period (also called "brain growth spurt"), there is a surge in brain growth characterized by neurogenesis, dendritic arborization, synaptogenesis and the migration of multiple neuronal populations (Bandeira et al., 2009; Dobbing and Sands, 1979) and some brain regions such as the frontal cortex and the hippocampus are very sensitive to ethanol (Gil-Mohapel et al., 2010; Olney et al., 2002b). Damage to neuronal circuits in these regions may lead to functional impairments in neurotransmission systems, thus triggering the emergence of hyperactivity (Goto and Grace, 2007).

Studies in rodents have suggested that impairments in the second messenger cAMP signaling pathway contribute to the hyperactivity phenotype in animals that are in a hypocatecholaminergic state (Paine et al., 2009; Pascoli et al., 2005; Russell, 2003). In the cAMP-regulated cascade (Medina, 2011b), the activation of adenylyl cyclase by the activation of dopamine, noradrenaline or NMDA receptors leads to the generation of intracellular cAMP that, in turn, activates protein kinases such as cAMP-dependent protein kinase A (PKA). PKA phosphorylates transcription factors such as CREB (cAMP response element binding protein), and SRF (serum response factor), leading to the expression of genes that modulate the neuronal excitability and plasticity within brain regions such as the frontal cortex and the hippocampus (Goto and Grace, 2007; Gurden et al., 1999, 2000). The deletion of SRF in dopaminoceptive neurons of mice causes a marked locomotor hyperactivity (Parkitna et al., 2010). The PKA inhibition within the medial prefrontal cortex of rats produces inattention and hyperactivity (Paine et al., 2009). Interestingly, ethanol exposure during development can alter several key factors in the cAMP/PKA signaling pathway (Conway and Garbouzova, 1996; Kumada et al., 2010; Maas et al., 2005), with long-lasting effects. Neonatal ethanol exposure promotes a reduction in CREB phosphorylation in the adult mice hippocampus (Roberson et al., 2009) and in the visual cortex of ferrets (Krahe et al., 2009). The overexpression of SRF by a Sindbis viral vector long after the period of ethanol exposure restores the ocular dominance plasticity in the visual cortex of a ferret model of FASD (Paul et al., 2010). The use of pharmacological or molecular tools to strengthen this signaling pathway opens up a great therapeutic possibility. Particularly, vinpocetine, a derivative of the Vinca minor alkaloid vincamine, is a phosphodiesterase type 1 (PDE1) inhibitor that has been successfully used for the treatment of neurobehavioral problems observed in animal models of FASD (Filgueiras et al., 2010; Krahe et al., 2009; Medina et al., 2006; Medina, 2011b). The PDE1 inhibition prevents the breakdown of cAMP to 5′-AMP, maintaining activation of protein kinases and transcription factors CREB and SRF (Krahe et al., 2009; Medina and Krahe, 2008; Paul et al., 2010).

Considering that impairments in the cAMP/PKA signaling system may contribute to the hyperactivity observed in FASD, here we investigated whether the acute administration of the PDE1 inhibitor vinpocetine ameliorates the hyperactivity observed in mice exposed to ethanol during the third trimester equivalent of human gestation. Additionally, we investigated whether the cAMP levels in the hippocampus and frontal cortex of adolescent mice are affected by neonatal exposure to ethanol.

2. Methods

2.1. Animal treatment

This study was conducted under institutional approval (protocol#: CEUA/040/2010) of the Universidade do Estado do Rio de Janeiro. All experiments were carried out in compliance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Subjects were Swiss mice that were bred and maintained in our laboratory on a 12:12 h light/dark cycle (lights on: 2:00, lights off: 14:00) at a constant temperature (22 °C). Access to food and water was unrestricted. Original breeding stock was obtained from Instituto Vital Brazil (Rio de Janeiro, RJ, Brazil).

From P2 to P8 (P1 = birth day), 104 animals from 9 litters received a single injection of ethanol (5 g/kg i.p., 25% in saline solution, ETOH group) every other day and 103 animals from 9 litters received an equivalent volume of saline solution (26 μL/g, SAL group) every other day. The treatment of entire litters with ethanol or saline was chosen based on previous data obtained in our laboratory which show that the mortality rate of ethanol-treated pups using this protocol is significantly lower than that of pups from litters in which half of the animals receive ethanol and the other half receive saline (Supplementary Material, A). The dose of ethanol was chosen based on previous studies (Filgueiras et al., 2009), which show that it generates blood ethanol concentrations (BECs) within the range that a human fetus would be exposed to after maternal ingestion of a moderate to heavy dose of ethanol (Eckardt et al., 1998). Treatment on alternate days was chosen since it mimics 'binge' drinking in humans, which is associated with severe neurobehavioral deficits (Maier and West, 2001). In order to minimize the risk of injury to internal organs, a 28-gauge needle was carefully inserted to just penetrate the abdominal wall and reach the peritoneal cavity. Leakage from the injection site was minimized by slowly withdrawing the needle from the abdominal cavity.

At weaning (P21), animals from the same litter were separated by sex and housed in groups of 2–5 mice by cage. From the initial sample of mice treated with ethanol or saline, only 149 (80 ethanol-injected and 69 saline-injected) were used for the behavioral analysis. The other 58 animals (24 ethanol-injected and 34 saline-injected), which were used in other studies (ETOH: $n = 11$; SAL: $n = 29$) or died (ETOH: $n = 13$, 12.5% mortality rate; SAL: $n = 5$, 4.9% mortality rate) during treatment, were considered only to estimate the mortality rate after ethanol or saline treatment. The mortality rate was calculated separately for each group by the number of animals that died until P30/total number of animals injected at P2. At P30, the animals were randomly assigned within each litter to receive treatment with vinpocetine (Vp) 20 mg/kg (i.p., in dimethylsulfoxide, DMSO, 0.5%, w/v), Vp10 mg/kg, or an equivalent volume of DMSO. Accordingly, we had 6 treatment groups: SAL + DMSO (14 females and 19 males), SAL + Vp10 mg (8 females and 8 males), SAL + Vp20 mg (10 females and 10 males), ETOH + DMSO (11 females and 13 males), ETOH + Vp10 mg (13 females and 14 males), ETOH + Vp20 mg (14 females and 15 males). Vinpocetine and DMSO were purchased from Sigma–Aldrich (St. Louis, MO). Injections were carried out 4 h before the behavioral test. This time-interval was chosen because it is close to the peak of increase in cAMP levels induced by vinpocetine administration in mice (unpublished data). Furthermore, we have demonstrated that vinpocetine 4 h before Morris water maze testing improved learning and memory deficits in rats early exposed to ethanol (Filgueiras et al., 2010).

2.2. Open field test

The open field arena consisted of a polypropylene box (37.6 cm × 30.4 cm × 17 cm) in which the floor was divided into 16 same-sized rectangles (7.6 cm × 9.4 cm), 12 peripheral and 4 central. The experiments were conducted under bright white light illumination during the dark part of the daily cycle, 1–2 h after its onset. Each mouse was individually placed in the center of the arena. Behaviors in the open field were recorded for 10 min (divided into 1 min intervals) with an overhead video camera. At the end of the session, the floor and walls were washed with odorless liquid soap, rinsed thoroughly with tap water and dried with a disposable paper towel. Recorded images of the tests were used to analyze behavior. The observer was blind regarding the experimental treatment of the animals. The ambulation was quantified on the basis of the number of rectangles crossed by the animals (Filgueiras et al., 2009). Mice had to place all four legs on a given rectangle for a crossing to be counted. The following ambulation variables were evaluated: ambulation in the center (C), ambulation in the periphery (Pe), C/Pe ratio and total ambulation (C + Pe). In addition, considering that direct comparisons between the activity in the center and in periphery can be influenced by the fact that the number of rectangles in the periphery is greater than that in the center, the rectangles crossed in the center and in the periphery were respectively divided by 4 (C/4) and 12 (Pe/12).

2.3. Blood ethanol concentration (BEC)

A separate group of mice was injected with ethanol or saline as described above. One or 2 h after the second injection (at P4), animals were decapitated and blood was collected (ethanol – 1 h: $n = 13$, 2 h: $n = 9$; saline – 1 h: $n = 10$, 2 h: $n = 6$). Blood was centrifuged at 2000 rpm for 5 min and the supernatant stored at 4 °C until assayed. BEC was assessed using an enzymatic kit (Alcohol Reagent Set, Pointe Scientific Inc., Michigan, USA) in accordance with the manufacturer's recommendations.

2.4. cAMP levels

After the test, 60 animals (at least 7 per group) were sacrificed by cervical dislocation. Frontal cerebral cortices (approximately the rostral third of the cerebral

Table 1
Mean litter weights (g).

	Postnatal day			
	P2	P4	P6	P8
SAL	1.9 ± 0.9	2.7 ± 0.1	3.6 ± 1.6	4.6 ± 0.3
ETOH	2.1 ± 1.9	2.9 ± 0.1	3.8 ± 1.8	4.7 ± 0.2

Values represent means ± SEM.

wall) and hippocampi were immediately dissected and incubated for 1 h at 37 °C in minimum essential medium (MEM) buffered with 20 mM HEPES at pH 7.3 and containing 100 mM ascorbic acid, 100 mM pargyline and 0.5 mM Rolipram (Sigma Chemical Co., St. Louis, MO, USA). After incubation, the reaction was interrupted by the addition of TCA to 10% (final concentration). cAMP was purified by removing trichloroacetic acid and endogenous interfering compounds from supernatant solution, using an ion exchange column of AGSOW-X4 (200–400 mesh, hydrogen form, Bio-Rad, Rio de Janeiro, Brazil), previously washed and equilibrated with H_2O (Matsuzawa and Nirenberg, 1975). Cyclic AMP concentrations of purified samples were determined by a protein binding assay described previously (de Mello et al., 1982; Gilman, 1970). cAMP levels were normalized to protein levels, assayed by the Lowry method.

2.5. Statistical analyses

Data are compiled as means and standard errors. In order to minimize the influence of litter effects, for all analyses, we considered the average of values from male and female mice of the same litter instead of using individual values (Wainwright, 1998). Separate univariate analyses of variance (uANOVA) were performed for weight data at P30, for cAMP levels in each region (frontal cerebral cortex or hippocampus) and for comparisons involving ambulation in the center and in the periphery of the open field. Repeated measures analyses of variance (rANOVA) were performed for both body weight data (postnatal; day as the within-subjects factor) and open field data (time-interval as the within-subjects factor). Neonatal exposure (ETOH or SAL), treatment at P30 (Vp10 mg, Vp20 mg or DMSO) and gender were used as between-subject factors for both uANOVAs and rANOVAS. Regarding rANOVAs, for simplicity, we report results based only on the averaged univariate *F* tests. Whenever the sphericity assumption was violated, we used the Greenhouse–Geisser correction, which adjusts the degrees of freedom, in order to avoid Type I errors. Significance was assumed at the level of $P < 0.05$ (two-tailed). In all cases, individual group differences were evaluated post-hoc by Fisher's Protected Least Significant Difference (FPLSD).

3. Results

3.1. BEC, survival rate and body weight gain

In the ethanol-injected group, BEC 1 and 2 h after injection were 316.2 ± 9.6 and 321.4 ± 6.5 mg/dL, respectively. In the saline-injected group, these levels were 2.4 ± 3.1 and 7.0 ± 4.7 mg/dL. The majority of the animals survived saline and ethanol i.p. injections. Survival rates were 87.5% ($n = 91$) in the ethanol-injected group and 95.1% ($n = 98$) in the saline-injected group. The difference between groups did not reach statistical significance (Fisher's Exact Test, $P = 0.56$).

Offspring weights during the injection period are shown in Table 1. The mean litter weights increased significantly from P2 to P8 [rANOVA, $F(1.2,18.7) = 600.6$; $P < 0.001$]. From P2 to P8, no differences were observed between ethanol-injected and saline-injected groups regarding weight gain or absolute weight. At P30 (open field testing day), no differences in body weight were observed between ethanol (21.9 ± 0.6 g) or saline (21.6 ± 0.6 g) injected animals [uANOVA, $F(1,35) = 0.1$; $P = 0.75$]. The mean litter weights of males (22.7 ± 0.6 g) was significantly higher than that of females (20.8 ± 0.6 g) [uANOVA, $F(1,35) = 4.6$; $P < 0.05$]. There was no interaction between gender and neonatal treatment [uANOVA, $F(1,35) = 0.01$; $P = 0.94$].

3.2. Open field data

For all animals collapsed across conditions, the ambulation in the periphery (89.5 ± 3.7) was significantly greater than in the center (19.0 ± 1.3) [uANOVA, $F(1,148) = 768.2$; $P < 0.001$] confirming that mice avoid open areas (Prut and Belzung, 2003). When ambulation values were corrected for the corresponding number of rectangles in the periphery (7.5 ± 0.3) and in the center (4.8 ± 0.3), this difference remained significant [uANOVA, $F(1,148) = 730.1$; $P < 0.001$]. Considering that activity in the center has been largely used as an indicator of anxiety (Prut and Belzung, 2003), the ambulation in the center was analyzed separately.

Regarding total ambulation (C + Pe), treatment with vinpocetine significantly ameliorated the hyperactivity induced by early ethanol exposure in a dose-dependent way [rANOVA: Neonatal Treatment × Treatment at P30 interaction, $F(2,63) = 3.6$; $P < 0.05$]. As depicted in Fig. 1, the ambulatory activity of the ETOH + DMSO group was ∼29% higher than that of the SAL + DMSO group (FPLSD, $P < 0.05$), ∼45% higher than that of the SAL + Vp10 mg group (FPLSD, $P < 0.05$) and ∼49% higher than that of the ETOH + Vp20 mg group (FPLSD, $P < 0.01$). The dose-dependent amelioration of hyperactivity elicited by vinpocetine was evidenced by the fact that the ETOH + Vp20 mg group had an average locomotor activity similar to that of the SAL + DMSO group while, distinctively, the ETOH + Vp10 mg group did not differ from both the SAL + DMSO and the ETOH + DMSO groups. No significant differences were observed between SAL + Vp20 mg and ETOH + DMSO as well as between males and females ($P > 0.05$ in all pairwise comparisons).

For both ambulation in the center and C/Pe ratio data, increases in values were observed along the 10 time-intervals [rANOVA: ambulation in the center, $F(6.3,393.1) = 3.3$; $P < 0.01$ and C/Pe ratio, $F(3.6,120.1) = 2.7$; $P < 0.05$]. However, for these two variables, no differences were observed between groups. Furthermore, no effects or interactions regarding gender, neonatal exposure and treatment at P30 were observed. Taken together, these results suggest that the ethanol-injected mice are hyperactive while maintaining normal levels of anxiety. In addition, the treatment with vinpocetine did not differentially affect the anxiety levels of ethanol- or saline-injected animals.

Regarding ambulation in the periphery, the results were similar to those described for total ambulation (C + Pe) (Supplementary Material, B).

3.3. cAMP data

Considering that the vinpocetine treatment effectively ameliorated hyperactivity only at the 20 mg/kg dose, we did not conduct the cAMP assays on the vinpocetine 10 mg/kg samples. As expected, treatment with vinpocetine increased the levels of cAMP by approximately 60% both in the hippocampus [uANOVA: $F(1,21) = 69.8$; $P < 0.001$] and in the cortex [uANOVA: $F(1,21) = 43.8$; $P < 0.001$]. In the hippocampus, neonatal exposure to ethanol reduced cAMP levels [uANOVA, $F(1,21) = 63.9$; $P < 0.001$] and treatment with vinpocetine significantly restored cAMP levels [uANOVA, $F(1,21) = 9.1$; $P < 0.01$]. Accordingly, cAMP levels in the ETOH + DMSO group were significantly lower than those observed in both SAL + DMSO (∼33%) and ETOH + Vp20 mg (∼31%) groups, which, in turn, did not differ from each other (Fig. 2A). No significant differences were observed between males and females.

In the cortex, cAMP levels in saline-exposed animals (SALINE + DMSO and SAL + Vp20 mg combined sample) were ∼17% higher than those of the ethanol-exposed ones (ETOH + DMSO and ETOH + Vp20 mg combined sample) [uANOVA, $F(1,21) = 7.0$; $P < 0.05$]. However, no differences were observed between ETOH + DMSO and SAL + DMSO groups when they were analyzed separately (Fig. 2B). No significant differences were observed between males and females.

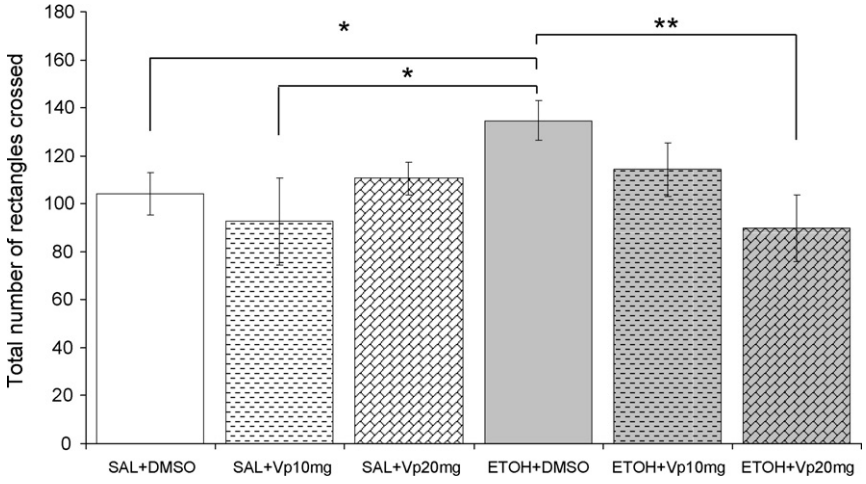

Fig. 1. Mean (±SEM) of total ambulation (C + Pe) in the open field of mice exposed to ethanol (ETOH) or saline (SAL) from P2 (P1 = birth day) to P8 and treated with vinpocetine 10 mg/kg (Vp10 mg), vinpocetine 20 mg/kg (Vp20 mg) or vehicle (DMSO) at P30. Note that the neonatal exposure to ethanol increases locomotor activity in animals treated with vehicle solution and that the treatment with 20 mg/kg of vinpocetine restores the locomotor activity to the control levels. FLSD: $^{*}P < 0.05$; $^{**}P < 0.01$.

4. Discussion

In the present study, we show that ethanol exposure during the third trimester equivalent of human gestation significantly increases locomotor activity in the open field. This result is in accordance with other studies in rodents exposed to ethanol during this period. Importantly, in these studies, the hyperactivity was described during the dark period irrespective of whether the animals were tested under dim red light (Melcer et al., 1994; Riley et al., 1993), bright light illumination (Slawecki et al., 2004) or with the lights off (Thomas et al., 2001). We also show that ethanol reduces cAMP levels and that the inhibition of the phosphodiesterase type 1 by vinpocetine significantly ameliorates hyperactivity and restores cAMP levels to control levels. Importantly, vinpocetine treatment was carried out long after the period of ethanol exposure, in a period equivalent to infancy/adolescence in humans. Our findings may be relevant from a clinical standpoint since they open up the possibility for treating juveniles when prevention fails.

During the brain growth spurt, ethanol triggers massive apoptotic neurodegeneration (Ikonomidou et al., 2000; Olney et al., 2002a). It has been assumed that neuronal loss is the main cause of reduced brain mass and lifelong neurobehavioral disturbances resulting from early ethanol exposure (Han et al., 2005; Medina, 2011a; Wozniak et al., 2004). Particularly, the locomotor hyperactivity observed in rodents exposed to ethanol during the brain growth spurt has been associated with an increase in neuronal death in cortex and hippocampus (Ieraci and Herrera, 2006). However, in addition to apoptotic neurodegeneration, early ethanol exposure may lead to persistent impairments in the function of surviving neurons (Medina, 2011a). Our finding that neonatal ethanol exposure reduced pubertal cAMP levels corroborates the idea that the cAMP/PKA signaling cascade may present long-lasting impairments in animals exposed to ethanol during the brain growth spurt. In addition, that vinpocetine restores cAMP levels in ethanol-exposed mice and ameliorates ethanol-induced hyperactivity suggests that an impairment in the second messenger cAMP signaling pathway plays a key role in generating the hyperactivity phenotype observed in FASD animal models (Paine et al., 2009; Pascoli et al., 2005; Russell, 2003).

Intracellular levels of cAMP are determined by the balance between its synthesis and breakdown. It is synthesized from ATP by adenylyl cyclase in response to hormones, neurotransmitters and various environmental stimuli, and it is broken down by PDEs (Lugnier, 2006). PDE1 is significantly expressed in neurons of the hippocampus and cortex (Lugnier, 2006), which suggests that this enzyme may control cAMP levels in areas that are markedly affected by ethanol exposure during the brain growth spurt (Gil-Mohapel et al., 2010; Olney et al., 2002a). Considering that the cAMP/PKA signaling system is involved in the control of a variety of cellular processes related with metabolism, gene transcription and neurotransmission, it is difficult to clearly identify the mechanism(s) through which the cAMP/PKA cascade and the ethanol-induced hyperactivity are linked.

One possibility is related to the fact that cAMP is a critical second messenger involved in catecholaminergic transmission and exerts its effects mainly through the PKA (Missale et al., 1998). Of note, PKA plays a key role in the control of the catalytic activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in the catecholamine biosynthesis. PKA acts by phosphorylating TH (Zigmond et al., 1989) or CREB, which is the major transcript factor for TH gene (Lewis-Tuffin et al., 2004). In the rat brain, the inhibition of PDE stimulates TH activity (Kehr et al., 1985) and increases the release of noradrenaline and dopamine in vitro (Schoffelmeer et al., 1985; Yamashita et al., 1997). In addition, in spontaneously hypertensive rats (SHR), a widely studied model for ADHD has demonstrated a reduced expression of TH (King et al., 2000; Wu et al., 2010). Based on this evidence, it is possible that the impairment in the cAMP/PKA cascade contributes to the reduction in the catecholaminergic function that, in turn, is strongly associated with the hyperactivity phenotype (O'Malley and Nanson, 2002).

In addition to the well-documented role of catecholaminergic dysfunction, other factors such as a deficient ATP production may play role in the pathophysiology of hyperactivity (Russell et al., 2006). Interestingly, the administration of vinpocetine increases ATP levels in the rat's cortex (Rosdy et al., 1976) and in astrocyte cultures (Gabryel et al., 2002). Finally, PDE1 inhibition caused by vinpocetine has also been reported to promote elevation of cGMP levels, which activates the cGMP-dependent protein kinase G (PKG) (Medina, 2011b). Although there are no studies associating cGMP levels and hyperactivity, it is not possible to discard that increased cGMP level significantly contributes to the vinpocetine-mediated amelioration of hyperactivity in ethanol-treated animals.

Some studies have proposed the use of PDE inhibitors as neuronal plasticity enhancers (Medina, 2011b; Navakkode et al., 2004; Puzzo et al., 2008). Neuronal plasticity entails functional changes in the efficacy of excitatory and inhibitory connections (e.g., synaptic strength), structural changes in the shape and size of synapses and in the physical connectivity of networks. Accordingly, vinpoce-

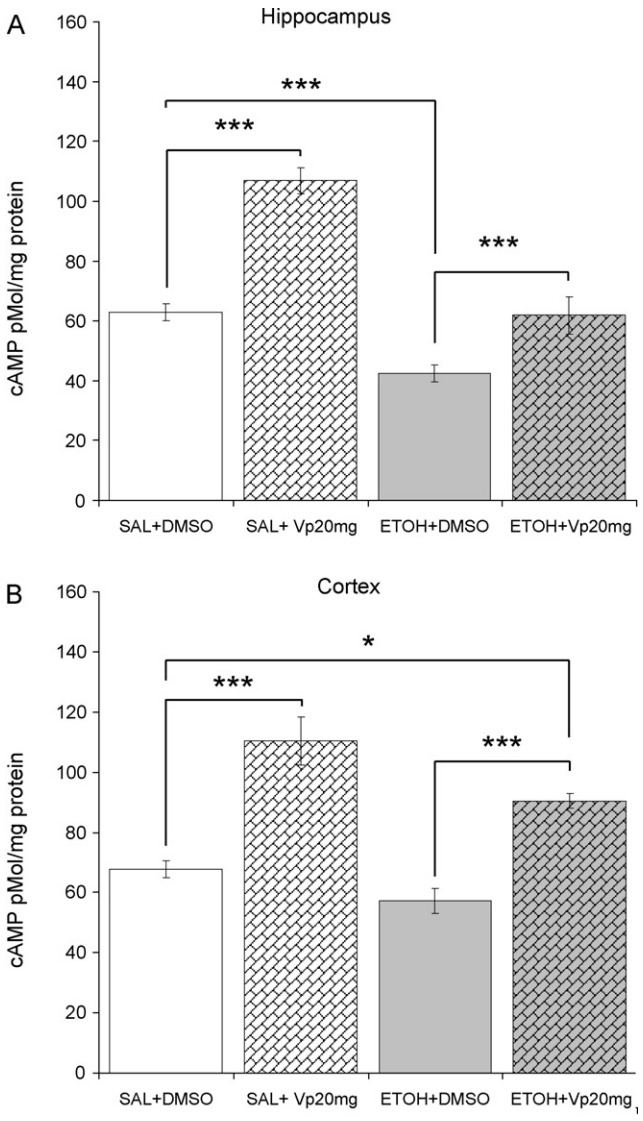

Fig. 2. Mean (±SEM) of cAMP levels in the hippocampus (A) and in the cortex (B) of mice exposed to ethanol (ETOH) or saline (SAL) from P2 (P1 = birth day) to P8 and treated with vinpocetine 20 mg/kg (Vp20 mg) or vehicle (DMSO) at P30. The treatment with vinpocetine increase cAMP levels both in the hippocampus and in the cortex. In the hippocampus, ethanol elicited a decrease in cAMP levels, which was restored by vinpocetine treatment. FLSD: $^{*}P < 0.05$; $^{***}P < 0.001$.

tine treatment has been shown to facilitate long-term potentiation (Molnar et al., 1994), to enhance the structural dynamics of dendritic spines (Lendvai et al., 2003), to improve learning/memory in rats (DeNoble, 1987) and to enhance performance on cognitive tests in humans (Kidd, 1999). It has been suggested that altered expression of genes, which results in reduced plasticity in the brain, triggers molecular mechanisms responsible for the development of psychopathological conditions involving hyperactivity, such as ADHD (Banaschewski et al., 2010; Jensen et al., 2009; Parkitna et al., 2010; Rapoport and Gogtay, 2008; Tsai, 2007). Interestingly, several lines of evidence suggest that deficits in neuronal plasticity underlie some of the neurobehavioral problems observed in FASD (Filgueiras et al., 2010; Krahe et al., 2009; Medina and Krahe, 2008; Paul et al., 2010; Puglia and Valenzuela, 2010). Taken together, these data suggest that neuronal plasticity deficits may also contribute to the hyperactivity observed in FASD and ADHD and, in this sense, the restorative effects of vinpocetine could be associated with its plasticity boosting properties.

It is important to note that the restorative effects of vinpocetine in FASD models have been demonstrated only for short periods after treatment (Filgueiras et al., 2010; Krahe et al., 2009; Medina et al., 2006). Therefore, whether the amelioration of the neurobehavioral deficits induced by ethanol during development is persistent remain to be investigated. Further studies in animal models of FASD are also required to verify if the treatment with vinpocetine has beneficial effects on the other two core features of ADHD: inattention and impulsivity. This issue is particularly important since it was found that although children with FASD display more behavioral problems and difficulties with attention and hyperactivity/impulsivity than typical children, attention deficits tend to account for more problems than hyperactive symptoms (Kodituwakku et al., 2006). Additionally, the ADHD medication may be less effective in treating the inattention symptom cluster in the FASD population (Doig et al., 2008). Another question that should be considered is that vinpocetine is already used (as Cavinton©) in some countries to treat cerebrovascular-related diseases without showing significant side effects at doses ranging from 15 to 45 mg per day (Kidd, 1999). Therefore, the safety and availability of this drug make this compound a promising agent for future clinical studies.

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Contributors

The author Claudio C Filgueiras designed the study, wrote the protocol and was responsible for the overall coordination and administration of the project. Alex C. Manhães and Yael Abreu-Villaça consulted on study design, interpretation of results and manuscript preparation. Fernanda Nunes and Kélvia Ferreira-Rosa gathered necessary behavioral data and participated in the initial draft of manuscript. Maurício dos S. Pereira and Regina C. Kubrusly gathered necessary biochemical data. All authors contributed to the final manuscript and have approved the final manuscript.

Conflict of interest

No conflict declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.drugalcdep.2011.05.024.

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